Effects of Experimental Heart Failure on Atrial Cellular and Ionic Electrophysiology

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Background—Congestive heart failure (CHF) is frequently associated with atrial fibrillation (AF), but little is known about the effects of CHF on atrial cellular electrophysiology.

Methods and Results—We studied action potential (AP) properties and ionic currents in atrial myocytes from dogs with CHF induced by ventricular pacing at 220 to 240 bpm for 5 weeks. Atrial myocytes from CHF dogs were hypertrophied (mean±SEM capacitance, 89±2 pF versus 71±2 pF in control, n=160 cells per group, P<0.001). CHF significantly reduced the density of L-type Ca$^{2+}$ current ($I_{\text{Ca}}$) by ≈30%, of transient outward K$^{+}$ current ($I_{\text{to}}$) by ≈50%, and of slow delayed rectifier current ($I_{\text{Kr,slow}}$) by ≈30% without altering their voltage dependencies or kinetics. The inward rectifier, ultrarapid and rapid delayed rectifier, and T-type Ca$^{2+}$ currents were not altered by CHF. CHF increased transient inward Na$^{+}$/Ca$^{2+}$ exchanger (NCX) current by ≈45%. The AP duration of atrial myocytes was not altered by CHF at slow rates but was increased at faster rates, paralleling in vivo refractory changes. CHF created a substrate for AF, prolonging mean AF duration from 8±4 to 535±82 seconds (P<0.01).

Conclusions—Experimental CHF selectively decreases atrial $I_{\text{to}}$, $I_{\text{Ca}}$, and $I_{\text{Kr,slow}}$, increases NCX current, and leaves other currents unchanged. The cellular electrophysiological remodeling caused by CHF is quite distinct from that caused by atrial tachycardia, highlighting important differences in the cellular milieu characterizing different clinically relevant AF substrates. (Circulation. 2000;101:2631-2638.)

Key Words: ion channels ◼ biophysics ◼ electrophysiology

Congestive heart failure (CHF) is a common cause of clinical atrial fibrillation (AF), but how it causes the arrhythmia is unclear. Atrial diseases associated with increased atrial pressure are accompanied by abnormal action potential (AP) properties in humans. Data regarding the effects of CHF on atrial ionic currents are limited. Atrial myocytes from hearts with terminal failure showed decreased L-type Ca$^{2+}$ current ($I_{\text{Ca}}$) compared with relatively normal hearts. Koumi et al noted reduced atrial inward rectifier current ($I_{\text{Kr,slow}}$) in patients with CHF; however, Le Grand et al found no change in $I_{\text{Kr,slow}}$ in dilated human atria, whereas $I_{\text{Ca}}$ and transient outward K$^{+}$ current ($I_{\text{to}}$) were reduced. These data are limited by a wide range of heart diseases and variable drug therapy. Many patients also had AF, which can directly alter atrial ionic properties. Thus, it is difficult to separate CHF-induced ionic abnormalities in patients from those caused by underlying heart disease, concomitant drug therapy, or AF. Rapid ventricular pacing has been used to study experimental CHF-induced ventricular ionic current changes; however, the ionic changes caused by experimental CHF at the atrial level are unknown and were the object of the present study.

Methods

Preparation of Animal Model

To produce CHF, the right ventricle was stimulated at 240 bpm for 3 weeks, followed by 2 weeks at 220 bpm. On study days, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV load, 29.25 mg·kg$^{-1}$·h$^{-1}$ infusion), and a median sternotomy was performed. Right atrial (RA) appendage effective refractory period (ERP) was measured with 15 basic (S$_1$) stimuli, followed by a premature (S$_2$) stimulus applied with 5-ms decrements (ERP=longest S$_1$/S$_2$ failing to capture, mean of 3 determinations). S$_1$ and S$_2$ were twice-threshold, 2-ms pulses. AF was induced 10 times by burst pacing (10-Hz, 2-ms pulses, 4× threshold currents), and mean duration was noted. If persistent AF requiring cardioversion occurred on 2 occasions, further AF inductions were not performed.

Hearts were excised and atrial cells isolated from RA pectinate muscles as previously described. In vivo studies were performed in the first 8 control and 18 CHF dogs. Because performing both in vivo and in vitro studies in each dog greatly prolonged the experiments, subsequent dogs were used for in vitro study only, after it had been verified that in vitro results were similar whether or not they were preceded by in vivo study.

Cellular Electrophysiology

Cells were isolated from 28 control and 28 CHF dogs. Cells in a 1-mL bath were superfused (3 mL/min, 35°C, or for the ultrarapid
Tight-seal patch-clamp was used to record currents (voltage-clamp mode) and APs (current-clamp mode). Electrode resistances were 1 to 2 MΩ for current recording and 3 to 5 MΩ for AP recording. Cells with normal resting potentials (negative to −70 mV) were selected for AP recording after it had been verified that resting potential was not altered by CHF. APs (elicited by 2-ms twice-threshold pulses) were analyzed at steady state at each frequency. Recordings were low-pass–filtered at half the sampling frequency (2 kHz for \(I_h\) and slow delayed rectifier current [\(I_{kr,d}\)], and 10 kHz for APs, \(I_{ca},\) \(I_{kr},\) and \(I_{kca,cv}\)).

Junction potentials (6 to 10 mV) were corrected for AP recordings only. Seal resistance averaged 8.6 ± 1.2 GΩ. Series resistance and capacitive time constant averaged 7.6 ± 0.3 MΩ and 506 ± 13 μs before and 1.6 ± 0.04 MΩ and 122.4 ± 6.2 μs after compensation, respectively. The mean maximum series resistance voltage drop was ∼3 mV. Similar numbers of cells from each heart were studied with each protocol. Cell capacitance (the time integral of capacitance divided by the voltage drop during 5-mV steps) was increased in CHF cells (89.2 ± 1.9 versus 70.5 ± 1.5 pA/fF in control, \(n=160\) each, \(P<0.001\)), so currents are presented in terms of densities.

Western Blot Analysis of NCX Expression

Western blot analysis of NCX expression has been used to evaluate ventricular NCX changes in CHF.\(^{11}\) To evaluate NCX protein expression, fast-frozen left atrial samples were placed on ice in radioimmunoprecipitation (RIPA) buffer containing Igepal 1%, sodium deoxycholate 0.5%, SDS 0.1%, β-mercaptoethanol 10 mmol/L, sodium orthovanadate 100 mmol/L, aprotinin 1 μg/mL, leupeptin 1 μg/mL, and PMSF 1 mmol/L in PBS. Samples were then minced and incubated/rotated (1 hour, 4°C). The homogenate was then centrifuged at 4°C and the supernatant stored at −70°C. Protein concentrations were measured by Bradford assay.

Solubilized membrane proteins were fractionated on 7.5% SDS-polyacrylamide gels at 200 V for 60 minutes, then transferred electrophoretically to Immobilon-P PVDF membranes (Millipore) in 25 mmol/L Tris base, 192 mmol/L glycine, and 5% methanol (18 hours, 0.07 A). Membranes were blocked with 5% nonfat dry milk (NFDM) in TBS (Tris-HCl 50 mmol/L, NaCl 500 mmol/L; pH 7.5) containing 0.05% Tween-20 (TTBS) (2 hours, room temperature), and then incubated overnight (4°C) with NCX antibody (Research Diagnostics; 1:1000 in TTBS with 1% NFDM). Membranes were then washed 3 times in TTBS and reblocked in 1% NFDM/TTBS for 10 minutes, followed by incubation with peroxidase-conjugated goat anti-mouse IgM (1:6000) in 5% NFDM/TTBS (30 minutes) and 3 TTBS washes. Antibody was detected with Western blot Chemiluminescence Reagent Plus (NEN Life Science Products). Band density was quantified with Quantity One software (PDQ) incorporating background subtraction algorithms. Total NCX density was calculated as the sum of the densities of each of the 3 isoforms.\(^{16}\)

Statistical Analysis

Group data are presented as mean ± SEM. Nonlinear curve fitting was performed with Clampfit in pCLAMP6 (Axony, San Diego, CA). Nonpaired \(t\) tests (2-tailed) were used for statistical comparisons between control and CHF cells.

Results

In Vivo and AP Findings

All dogs with CHF had increased RA (12 ± 1 versus 3.0 ± 0.3 mm Hg, \(P<0.01\)) and left ventricular (19 ± 1 versus 2 ± 1 mm Hg, \(P<0.01\)) diastolic pressures, evident ascites and pulmonary congestion, and increased respiratory rates (56 ± 2 versus 24 ± 1 breaths per minute, \(P<0.001\)). Atrial rates in CHF dogs averaged 139 ± 5 bpm with and 138 ± 4 bpm without ventricular pacing, versus 138 ± 6 bpm in controls. Mean AF duration was 8 ± 4 seconds in controls, versus 535 ± 82 seconds in CHF dogs (\(P<0.01\)), with persistent AF.
Resting potential averaged \(-74.0 \pm 0.9\) and \(-72.4 \pm 0.4\) mV in 20 control and 20 CHF myocytes used for AP analysis, respectively (P=NS). AP amplitude (1 Hz) averaged 118.8 \pm 4.1 mV in control and 116.0 \pm 3.8 mV in CHF myocytes (P=NS). CHF prolonged AP duration (APD) increasingly as frequency increased (Figure 1). APDs in isolated cells paralleled ERP in vivo (eg, at 3 Hz, APD\(_{90}\)=138 \pm 4\) ms in controls and 145 \pm 6\) ms in CHF; ERP=131 \pm 8\) ms in controls and 149 \pm 7\) ms in CHF; at 6 Hz, APD\(_{90}\)=97 \pm 3\) ms in controls and 117 \pm 6\) ms in CHF; ERP=93 \pm 4\) ms in controls and 122 \pm 7\) ms in CHF). We did not observe delayed afterdepolarizations (DADs) during AP recording.

Changes in K\(^+\) Currents

\(I_K\) was decreased by CHF (Figure 2A through 2C). CHF did not alter the form of the I-V relation (Figure 2D) or voltage dependence (Figure 2E) or the kinetics (Figure 2F) of \(I_K\) activation and inactivation. \(I_{Kr}\) reactivation (Figure 2G) was monoeXponential (time constants for control versus CHF were 28.1 \pm 0.3 versus 30.1 \pm 0.5 \) ms at -70 mV, 59.6 \pm 8.5 versus 65.3 \pm 2.4 \) ms at -60 mV, and 93.4 \pm 11.9 ms at -50 mV; n=7 per group, P=NS) and, along with frequency dependence (Figure 2H), was unaffected by CHF.

To study \(I_K\), currents were recorded as shown in Figure 3 10 minutes after membrane rupture and then again at least twice at 10-minute intervals to exclude cells with >10% rundown over 20 minutes. Dofetilide (1 \(\mu\)mol/L) was then added to block \(I_K\). The drug-resistant component (\(I_{Ks}\), Figure 3A and 3B) was reduced by CHF. CHF reduced both step (Figure 3C) and tail (Figure 3D) current densities by >30\%. The voltage dependence of \(I_{Ks}\) activation (tail-current analysis, Figure 3E) was not altered by CHF (mean \(V_{1/2}\)=21.3 \pm 2.5\) mV in CHF and +22.1 \pm 2.5\) mV in control; n=15 per group), nor were the kinetics of \(I_{Ks}\) activation (Figure 3F).
Examples of dofetilide-sensitive \( I_{\text{Kr}} \) in control and CHF cells are shown in Figure 4A and 4B. \( I_{\text{Kr}} \) density (Figure 4C) and activation voltage dependence (Figure 4D) were not changed by CHF. Neither \( I_{\text{K1}} \) nor \( I_{\text{Kur,d}} \) were altered by CHF, as shown in Figure 5.

**Changes in Ca\(^{2+}\) Currents**

Typical L-type \( I_{\text{Ca}} \) recordings in control and CHF cells are shown in Figure 6A and 6B. \( I_{\text{Ca}} \) density decreased significantly (Figure 6C) in CHF myocytes (eg, from \(-16.6\pm1.4\) to \(-11.5\pm0.1\) pA/pF at \(+10\) mV, \( n=35 \) cells each, \( P<0.01 \)), without any alteration in the form of the I-V curve (Figure 6D). CHF did not alter the voltage dependence of \( I_{\text{Ca}} \) inactivation or activation (Figure 6E). The time course of \( I_{\text{Ca}} \) inactivation was biexponential and voltage dependent but was not altered by CHF (Figure 6F). \( I_{\text{Ca}} \) recovery was similar in CHF and normal myocytes (Figure 6G), with monoexponential time constants averaging \( 28.6\pm1.5 \) and \( 30.9\pm3.5 \) ms for control and CHF, respectively. CHF similarly did not alter \( I_{\text{Ca}} \) frequency dependence (Figure 6H).

T-type \( I_{\text{Ca}} \) was separated from L-type by subtraction of currents recorded at a HP of \(-50\) mV from those at \(-90\) mV, as illustrated in Figure 7A and 7B. \( I_{\text{CaT}} \) was present in 40% of atrial myocytes (20 of 50 for both control and CHF). As shown in Figure 7C and 7D, CHF did not alter \( I_{\text{CaT}} \) density.

**NCX Expression**

NCX recordings in control and CHF cells are shown in Figure 8A. Substitution of Li\(^+\) for extracellular Na\(^+\) strongly suppressed inward currents, typical for the NCX.\(^{12,14}\) CHF substantially increased the density of NCX current (Figure 3).
Both with tricuspid insufficiency and chronic mitral regurgitation, APD was not substantially altered in dogs with atrial disease and/or dilation. Similar to our observations been reported in patients and experimental animals. It is well recognized that CHF frequently causes AF in humans. Alterations in atrial cellular electrophysiology have been reported in dilated human atria. Atrial tachycardia reduces $I_K$, L-type $I_{Ca}$, and $I_K$, and increases NCX in atrial myocytes, without altering T-type $I_{Ca}$, $I_K$, or $I_{Kr,d}$. The atrial cellular electrophysiological substrate produced by CHF is quite different from that seen with atrial tachycardia–induced remodeling, although both experimental paradigms promote AF.

Comparison With Previous Studies of Cellular Electrophysiology Associated With Atrial Disease and Arrhythmias

It is well recognized that CHF frequently causes AF in humans. Alterations in atrial cellular electrophysiology have been reported in patients and experimental animals with atrial disease and/or dilation. Similar to our observations at slower rates, APD was not substantially altered in dogs with tricuspid insufficiency and chronic mitral regurgitation. Cats with endogenous cardiomyopathy had APDs that were either unchanged or (in more severe cases) increased in the left atrium. Decreases in $I_K$ and $I_{Kr}$ have been reported in dilated human atria. $I_K$ was unchanged in dilated atria but reduced in atria of patients with symptomatic CHF. Both $I_K$ and the sustained outward current are reduced in patients with CHF. It is clear that atrial pathology affects cellular electrophysiology and that the latter changes contribute to arrhythmogenesis. However, the information about ionic alterations obtained from studies in human tissues is limited by various disease states, duration of disease, drug therapy, and numbers of patients with AF in each study. For example, in the study by Le Grand et al of ionic currents in patients with dilated atria, $25\%$ of patients also had AF. In the study by Van Wagoner et al of $K^+$ current changes in patients with AF, all the tissue samples were from patients with significant dilation of $\geq 1$ atria.

Sustained atrial tachycardia causes a substrate that supports AF and at the cellular level reduces APD and APD rate adaptation. Although CHF also produced a substrate for AF in the present study, APD was unchanged at slower rates and increased at faster rates. APD adaptation was reduced, but not as much as with atrial tachycardia. The differences in APD remodeling between the CHF and atrial tachycardia models are associated with differences in ionic current changes. Atrial tachycardia reduces $I_K$ by $\approx 70\%$ without affecting $I_K$. CHF reduced $I_K$ to a lesser extent (by $\approx 30\%$), significantly reduced $I_K$ by reducing $I_{Kr,d}$ (to the same extent as $I_K$), and increased the inward current carried by the NCX. NCX expression is not affected by rapid atrial pacing and we are not aware of previous studies of atrial NCX current in patients with CHF. In the atrial tachycardia model, strong reductions in $I_{Kr}$ largely account for decreases in APD and APD rate adaptation. In CHF cells, the $I_K$ reductions are offset by decreases in $I_K$ and increases in NCX, which probably accounts for the lack of APD alterations at slow rates. The attenuated rate adaptation caused by $I_K$ reduction may account for the increased APD at faster rates.

Comparison of Atrial Electrophysiological Abnormalities With Those in Ventricular Myocytes Caused by CHF

We are unaware of other studies of the impact of experimental CHF on atrial cellular electrophysiology. There is, however, an extensive literature regarding ventricular cellular abnormalities caused by CHF. Ventricular $I_K$ is decreased in dogs with pacing-induced CHF and in cardiomyopathic Syrian hamsters. Conflicting results have been reported for ventricular L-type $I_{Ca}$, with either a reduction or no change reported. Ventricular APD is generally prolonged by CHF. Ventricular $I_K$ is reduced in both experimental and clinical CHF. Ventricular NCX is increased by experimental CHF and probably plays an important role in APD prolongation and ventricular tachyarrhythmia promotion at the ventricular level.
Potential Significance
The APD decrease caused by atrial tachycardia plays an important role in leading to multiple-circuit reentrant AF. The lack of APD decrease in CHF cells suggests other mechanisms of AF promotion. Previous work from our laboratory suggests that structural changes, particularly interstitial fibrosis, cause important local conduction abnormalities that may play a central role in the reentrant substrate of CHF-related AF. The NCX increases that we noted in the present study may be quite important in atrial arrhythmia promotion by CHF. The NCX is known to carry transient inward currents that cause DADs and triggered activity. Stambler and coworkers have observed atrial tachycardias with properties of triggered arrhythmias in dogs with CHF, to which increased NCX could well be an important contributor. AF is readily induced in CHF dogs by atrial burst pacing but not by single extrastimuli. Therefore, atrial tachycardias caused by NCX-related triggered activity may be the trigger needed to initiate AF in the presence of the CHF-induced structural substrate. We did not observe triggered activity or DADs during AP recording in vitro. Additional conditions present in vivo in the setting of CHF, such as increased
catecholamines, tissue stretch, and metabolic abnormalities, may therefore be necessary to produce triggered activity.

Our results could have potential implications for understanding the effects of antiarrhythmic drug therapy on AF. Most antiarrhythmic drugs act by inhibiting cardiac ion channels, so any change in the relative importance of currents flowing during the AP could have a major impact on the action of antiarrhythmic drugs. For example, we have found dofetilide to be unusually effective in terminating AF in dogs with experimental CHF.25b This greater efficacy may be due to CHF-induced downregulation of atrial I\textsubscript{Ks}, leaving repolarization more dependent on I\textsubscript{Kr}.

The development of the atrial tachycardia model of AF was an important advance in studying the arrhythmia.23,26,27 It provided a reliable and reproducible animal model of AF, in which the cellular and ionic mechanisms underlying the arrhythmia substrate could be studied.7 The present study adds a new element to our understanding of the cellular electrophysiological basis of AF by showing that experimentally induced ventricular failure produces a substrate for AF with different AP andionic changes from those caused by atrial tachycardia. Both of these experimental paradigms are important for our understanding of the cellular basis of AF in humans. When AF begins in patients with CHF, the underlying cellular electrophysiological milieu is established by the ionic current abnormalities caused by CHF. Should AF persist, the resulting atrial tachycardia will cause further atrial cellular remodeling, superimposing tachycardia-induced changes on the underlying CHF substrate. This dynamic nature of the cellular substrate for AF is important to appreciate in order to understand the factors controlling the occurrence and perpetuation of the arrhythmia.

**Potential Limitations**

I\textsubscript{K} is very sensitive to isolation procedures,10 and therefore, changes in isolation technique could contribute to differences in I\textsubscript{K} when several groups are compared. To minimize possible effects of time-dependent changes in enzymes, isolation procedure, etc, animals from each group were studied concurrently in an alternating fashion. Rundown can be a problem in studies of I\textsubscript{K} and I\textsubscript{Ca}. All current protocols were applied in the same order for control and CHF cells. Cells with significant rundown (>10% over 20 minutes) were rejected.

We studied a specific animal model of CHF, which has a clinical counterpart in tachycardia-induced ventricular cardiomyopathies28 but which may be different in specific aspects of pathophysiology from other forms of clinical CHF. Furthermore, such issues as the rate of development of CHF, the neuroendocrine response, and the distribution of hemodynamic load may be important determinants of ionic changes. Ionic heterogeneity is significant in the canine atrium,29 and it is therefore essential to compare cells from the same region(s) in CHF dogs versus normal dogs. We studied cells from right atrial pectinate muscles to compare with previous studies of tachycardia-induced ionic remodeling performed with right atrial pectinate myocytes.7 Further work on regional variability in remodeling would be interesting but is outside the scope of this study.

**Conclusions**

We have found that experimental CHF promotes AF and causes discrete changes in atrial AP properties and currents. NCX overexpression may be particularly important by promoting the occurrence of arrhythmogenic afterdepolarizations. The differences between CHF-induced atrial cellular electrophysiological remodeling and that caused by atrial tachycardia point to the potentially variable ionic pathophysiology associated with different substrates for AF. These results are of fundamental importance for our understanding of basic atrial arrhythmia mechanisms and the response to antiarrhythmic drugs.

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